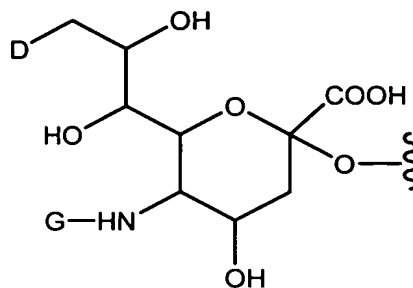**WHAT IS CLAIMED IS:**

1.   **1.**   A Granulocyte Colony Stimulating Factor peptide comprising the moiety:

2

3   wherein

4   D is a member selected from -OH and R^1-L-HN-;

5   G is a member selected from R^1-L- and -C(O)(C$_1$-C$_6$)alkyl;

6   R^1 is a moiety comprising a member selected a moiety comprising a straight-

7   chain or branched poly(ethylene glycol) residue; and

8   L is a linker which is a member selected from a bond, substituted or

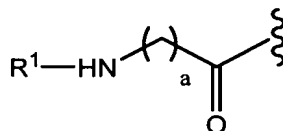9   unsubstituted alkyl and substituted or unsubstituted heteroalkyl,

10   such that when D is OH, G is R^1-L-, and when G is –C(O)(C$_1$-C$_6$)alkyl, D is

11   R^1-L-NH-.

1   **2.**   The peptide according to claim 1, wherein L-R^1 has the formula:
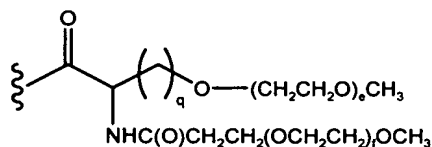

2

3   wherein
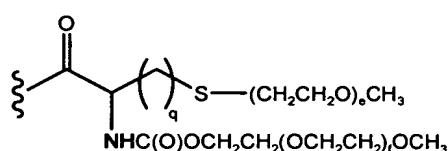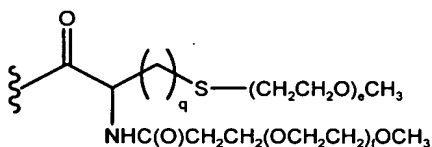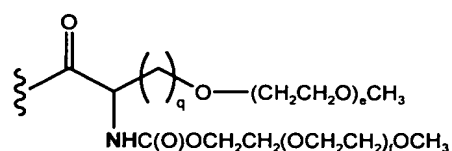
4   a is an integer from 0 to 20.

1   **3.**   The peptide according to claim 1, wherein R^1 has a structure that is a member

2   selected from:
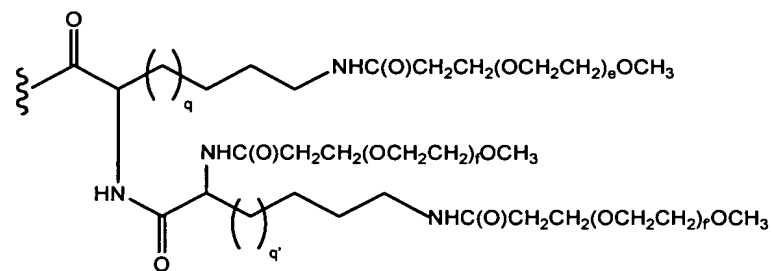

3

4   wherein

107

5   e and f are integers independently selected from 1 to 2500; and

6   q is an integer from 0 to 20.

1   **4.**   The peptide according to claim 1, wherein R^1 has a structure that is a member

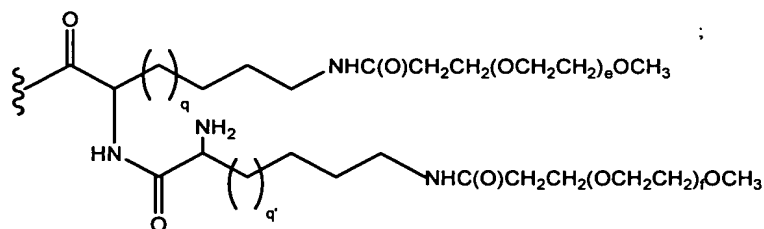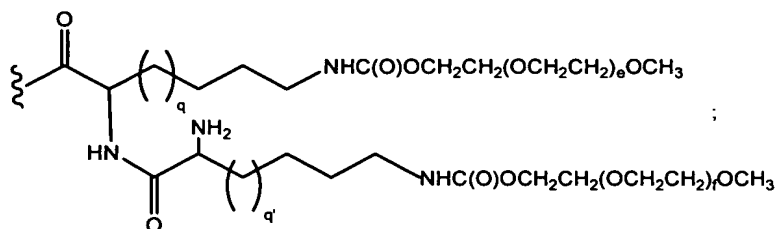2   selected from:

3

4   wherein

5   e, f and f' are integers independently selected from 1 to 2500; and

6   q and q' are integers independently selected from 1 to 20.

5. The peptide according to claim 1, wherein R^1 has a structure that is a member selected from:
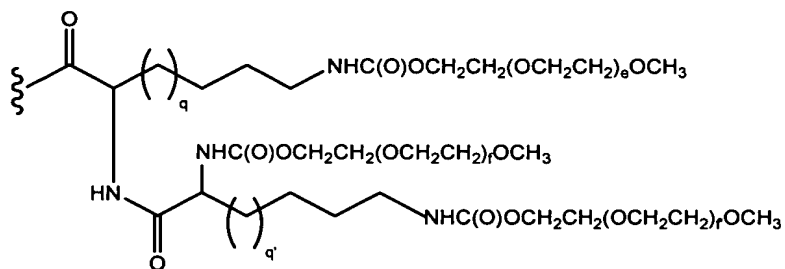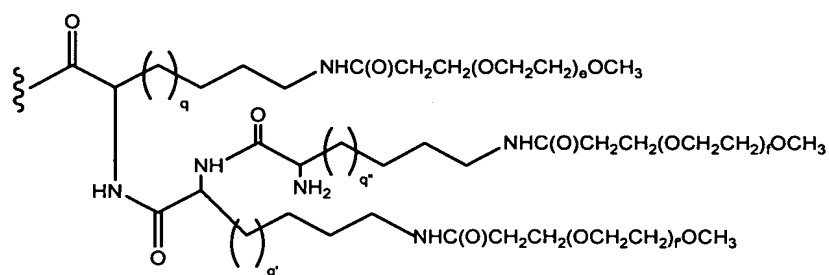

; and

wherein

e, f and f' are integers independently selected from 1 to 2500; and

q, q' and q''are integers independently selected from 1 to 20.

6. The peptide according to claim 1, wherein R^1 has a structure that is a member selected from:

$$\xi\text{—}C(O)CH_2CH_2(OCH_2CH_2)_eOCH_3 \quad ; \text{and}$$

$$\xi\text{—}C(O)OCH_2CH_2(OCH_2CH_2)_fOCH_3$$
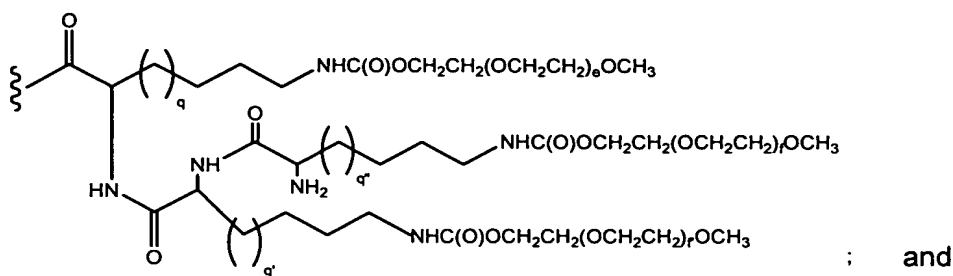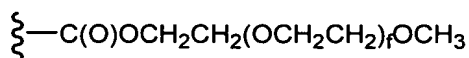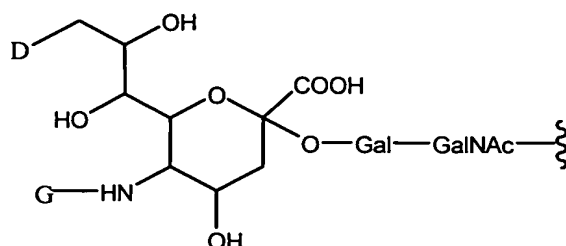
wherein

e and f are integers independently selected from 1 to 2500.

7. The G-CSF peptide according to claim 1, wherein said moiety has the formula:

.
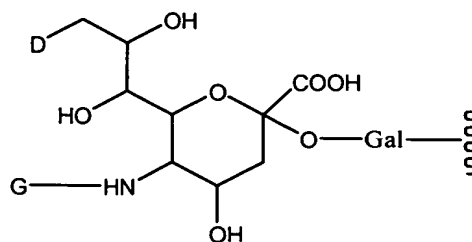
1   **8.**   The G-CSF peptide according to claim 1, wherein said moiety has the

2   formula:

3

1   **9.**   The G-CSF peptide according to claim 1, wherein said moiety has the

2   formula:

3

4   wherein

5   AA is an amino acid residue of said peptide.

1   **10.**   The G-CSF peptide according to claim **9,** wherein said amino acid residue is a

2   member selected from serine or threonine.

1   **11.**   The G-CSF peptide according to claim **1,** wherein said peptide has the amino

2   acid sequence of SEQ. ID. NO:1.

1   **12.**   The G-CSF peptide according to claim **11,** wherein said amino acid residue is

2   threonine at position 133 of SEQ. ID. NO:1.

1   **13.**   The peptide according to claim 1, wherein said peptide has an amino acid

2   sequence selected from SEQ. ID. NO:1 and SEQ ID NO:2.

1   **14.**   The G-CSF peptide according to claim **1,** wherein said moiety has the

2   formula:

```
        ⌠                              ╭ [[GlcNAc-(Gal)a]e- (Sia)j - (R)v ]r ⌉
  ∿∿∿   │(Fuc)i              Man      ╱
        │  |                     ╲  ╲ [[GlcNAc-(Gal)b]f- (Sia)k - (R)w ]s
§—AA——│——GlcNAc-GlcNAc-Man
        │  |                     ╱  ╱ [[GlcNAc-(Gal)c]g- (Sia)l - (R)x ]t
        │                 Man    ╲
        ⌡                         ╲ [[GlcNAc-(Gal)d]h- (Sia)m - (R)y ]u ⌡q
```
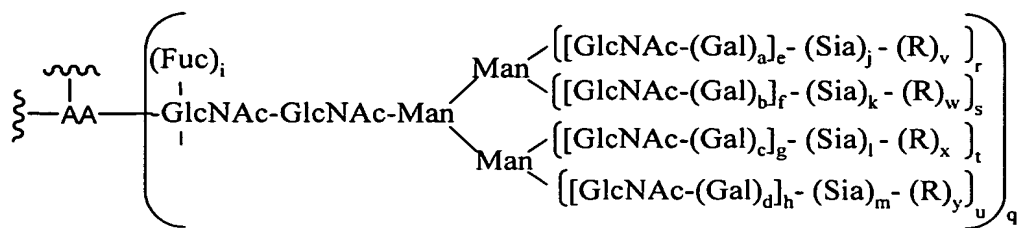
3

4   wherein

5   a, b, c, d, i, r, s, t, and u are integers independently selected from 0 and 1;

6   q is 1;

7   e, f, g, and h are members independently selected from the integers from 0 to

8   6;

9   j, k, l, and m are members independently selected from the integers from 0 and

10   100;

11   v, w, x, and y are independently selected from 0 and 1, and least one of v, w, x

12   and y is 1;

13   AA is an amino acid residue of said G-CSF peptide;

14   Sia-(R) has the formula:

15

16   wherein

17   D is a member selected from -OH and R^1-L-HN-;

18   G is a member selected from R^1-L- and -C(O)(C$_1$-C$_6$)alkyl;

19   R^1 is a moiety comprising a member selected a straight-chain or

20   branched poly(ethylene glycol) residue; and

21   L is a linker which is a member selected from a bond, substituted or

22   unsubstituted alkyl and substituted or unsubstituted heteroalkyl,

23   such that when D is OH, G is R^1-L-, and when G is –C(O)(C$_1$-C$_6$)alkyl,

24   D is R^1-L-NH-.
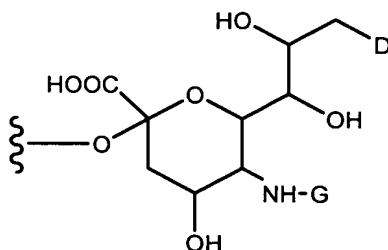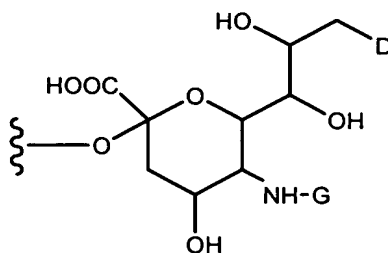

111

1   **15.**   The peptide according to claim 14, wherein said amino acid residue is an

2   asparagine residue.

1   **16.**   The peptide according to claim 1, wherein said peptide is a bioactive

2   Granulocyte Colony Stimulating Factor peptide.

1   **17.**   A method of making a G-CSF peptide conjugate comprising the moiety:

2

3   wherein

4   D is a member selected from -OH and R^1-L-HN-;

5   G is a member selected from R^1-L- and -C(O)(C$_1$-C$_6$)alkyl;

6   R^1 is a moiety comprising a member selected a straight-chain or branched

7   poly(ethylene glycol) residue; and

8   L is a linker which is a member selected from a bond, substituted or

9   unsubstituted alkyl and substituted or unsubstituted heteroalkyl,

10   such that when D is OH, G is R^1-L-, and when G is –C(O)(C$_1$-C$_6$)alkyl, D is

11 R^1-L-NH-,

12   said method comprising:

13   (a) contacting a substrate G-CSF peptide with a PEG-sialic acid donor moiety

14   having the formula:
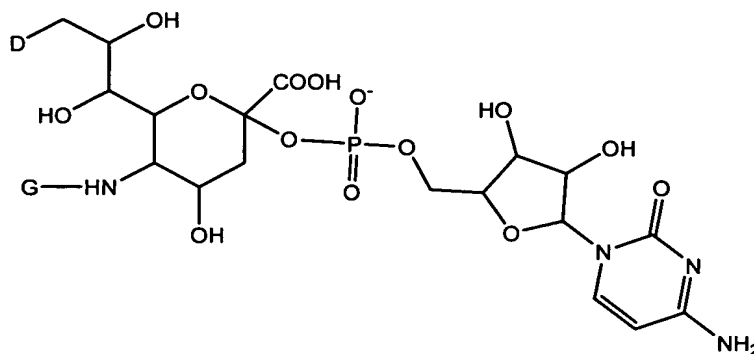
15

16   and an enzyme that transfers said PEG-sialic acid onto an amino acid

17   or glycosyl residue of said G-CSF peptide, under conditions

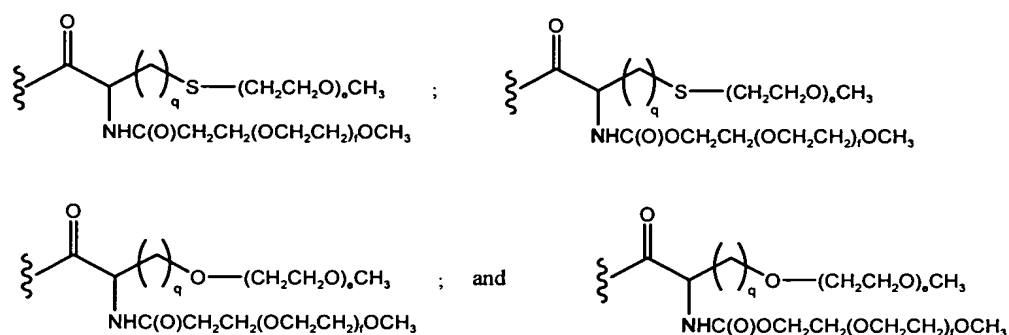18   appropriate for the transfer.

112

1   **18.**   The method according to claim 17, wherein L-R^1 has the formula:

2
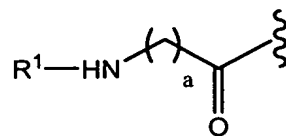
3   wherein

4   a is an integer from 0 to 20.

1   **19.**   The method according to claim 17, wherein R^1 has a structure that is a

2   member selected from:
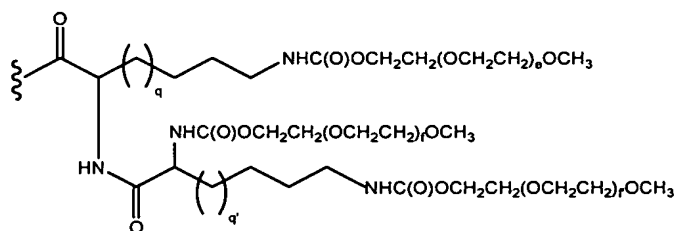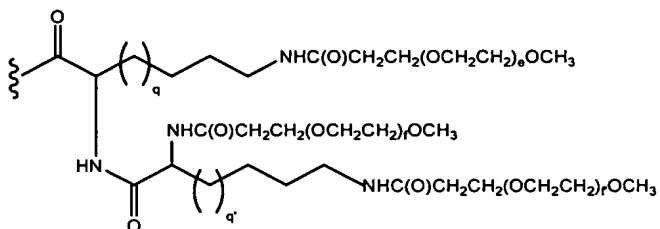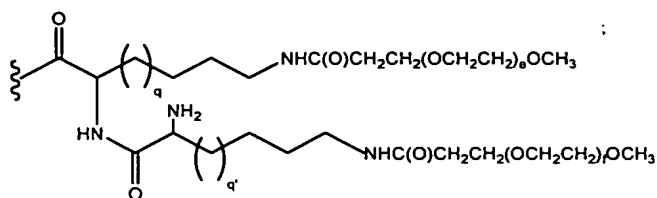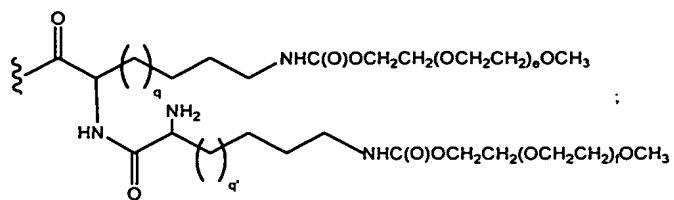

3

4   wherein

5   e and f are integers independently selected from 1 to 2500; and

6   q is an integer from 0 to 20.

1   **20.**   The method according to claim 17, wherein R^1 has a structure that is a

2   member selected from:

113

NHC(O)OCH₂CH₂(OCH₂CH₂)ₑOCH₃

NHC(O)OCH₂CH₂(OCH₂CH₂)ᵢOCH₃

;

NHC(O)CH₂CH₂(OCH₂CH₂)ₑOCH₃

NHC(O)CH₂CH₂(OCH₂CH₂)ᵢOCH₃

;

NHC(O)CH₂CH₂(OCH₂CH₂)ₑOCH₃

NHC(O)CH₂CH₂(OCH₂CH₂)ᵢOCH₃

NHC(O)CH₂CH₂(OCH₂CH₂)ᵢOCH₃

; and

NHC(O)OCH₂CH₂(OCH₂CH₂)ₑOCH₃

NHC(O)OCH₂CH₂(OCH₂CH₂)ᵢOCH₃

NHC(O)OCH₂CH₂(OCH₂CH₂)ᵢOCH₃

wherein

e, f and f' are integers independently selected from 1 to 2500; and

q and q' are integers independently selected from 1 to 20.

114

1 **21.** The method according to claim 17, wherein R^1 has a structure that is a
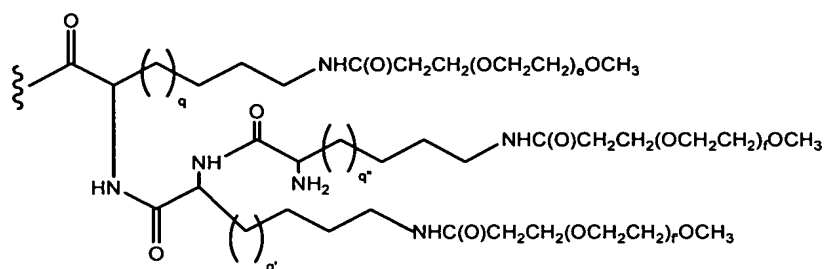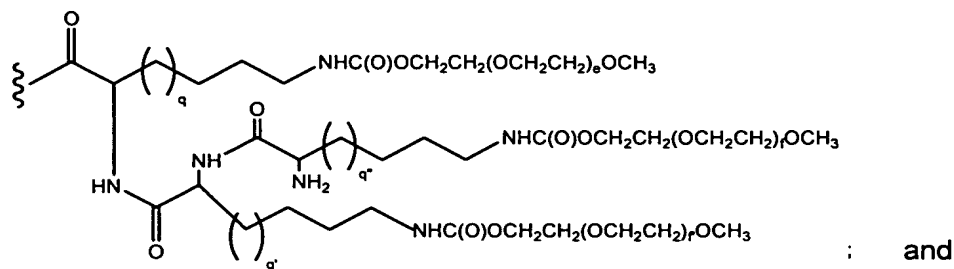2 member selected from:

; and

3
4 wherein
5   e, f and f' are integers independently selected from 1 to 2500; and
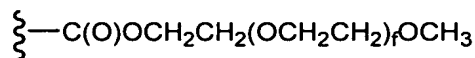6   q, q' and q''are integers independently selected from 1 to 20.

1 **22.** The method according to claim 17, wherein R^1 has a structure that is a
2 member selected from:

$$\xi—C(O)CH_2CH_2(OCH_2CH_2)_eOCH_3 \text{ ; and}$$

3
$$\xi—C(O)OCH_2CH_2(OCH_2CH_2)_fOCH_3$$

4 wherein
5   e and f are integers independently selected from 1 to 2500.

1 **23.** The method of claim **17**, further comprising, prior to step (a):
2   (b) expressing said substrate Granulocyte Colony Stimulating Factor
3 peptide in a suitable host.

1 **24.** The method of claim **17**, wherein said host is selected from an insect cell and a
2 mammalian cell.

1 **25.** A method of stimulating inflammatory leukocyte production in a mammal,
2 said method comprising administering to said mammal a peptide according to claim1.

115

1   **26.**   A method of treating infection in a subject in need thereof, said method

2   comprising the step of administering to the subject an amount of a peptide according

3   to claim 1, effective to ameliorate said condition in said subject.

1   **27.**   A pharmaceutical formulation comprising the Granulocyte Colony Stimulating

2   Factor peptide according to claim 1, and a pharmaceutically acceptable carrier.

1   **28.**   A method of refolding an insoluble recombinant granulocyte colony

2   stimulating factor (GCSF) protein, the method comprising the steps of:

3   (a)   solubilizing the GCSF protein; and

4   (b)   contacting the soluble GCSF protein with a buffer comprising a

5   redox couple to refold the GCSF protein, wherein the refolded GCSF protein is

6   biologically active.

1